Check List the journal of biodiversity data

NOTES ON GEOGRAPHIC DISTRIBUTION

Check List 14 (4): 613–617
https://doi.org/10.15560/14.4.613

PENSOFT

First record of the invasive coral *Oculina patagonica* de Angelis, 1908 (Cnidaria, Scleractinia) in the Gulf of Mexico

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Abstract

Colonies of coral with morphology similar to that of *Oculina patagonica* de Angelis, 1908 were found in the National Park Veracruz Reef System (NPVRS) along the coast of Veracruz, Mexico in the southern Gulf of Mexico. The identity of these colonies as *O. patagonica* was confirmed by morphological and molecular analyses. Here, we document the first records of *O. patagonica* in the Gulf of Mexico. This species is native to the Mediterranean Sea, and could have been accidentally introduced to the Gulf of Mexico through ballast water from ships. In the NPVRS, poor environmental conditions such as polluted waters with high sedimentation, and the capability of *O. patagonica* to adapt could have facilitated the establishment of this species in the Gulf.

Key words

Adaptation, coral distribution, dispersion, invasive species, establishment, polluted reefs, shipping transport.

Academic editor: Sergio N. Stampar | Received 18 October 2016 | Accepted 20 June 2018 | Published 3 August 2018

Citation: Colín García NA, Campos JE, Tello Musi JL, Perez España H, Carrara XC (2018) First record of the invasive coral *Oculina patagonica* (Cnidaria, Scleractinian) in the Gulf of Mexico. Check List 14 (4): 613–617. https://doi.org/10.15560/14.4.613

Introduction

Coral dispersion depends of water currents that transport coral larvae between reefs. However, anthropogenic activities have altered coral distributions and facilitated the expansion of invasive species (Crooks 2001). The invasive scleractinian coral *Oculina patagonica* de Angelis, 1908 was recorded for the first time in the Gulf of Genoa in 1966 (Zibrowius 1974), and expanded in the Mediterranean Sea, as a response to environmental change (Leydet and Helberg 2015). The introduction and invasion of new areas by corals has been recorded in *Oculina* (Brito et al. 2017) and other scleractinian corals, such as *Tubastraea* species which are native to the Indo-Pacific and have invaded the tropical western Atlantic (Cairns 2000, Ferreira et al. 2004) after being transported there by oil tankers (Creed et al. 2016).

Oculina patagonica can reproduce both sexually and asexually, at an early age, it has a high growth rate, and is resistant to extreme environmental conditions (Fine et al. 2001). These characteristics allow *O. patagonica* to grow and reproduce in various environmental conditions and have a wide distribution throughout the Mediterranean Sea and Atlantic Ocean (Zibrowius and Ramos 1983,

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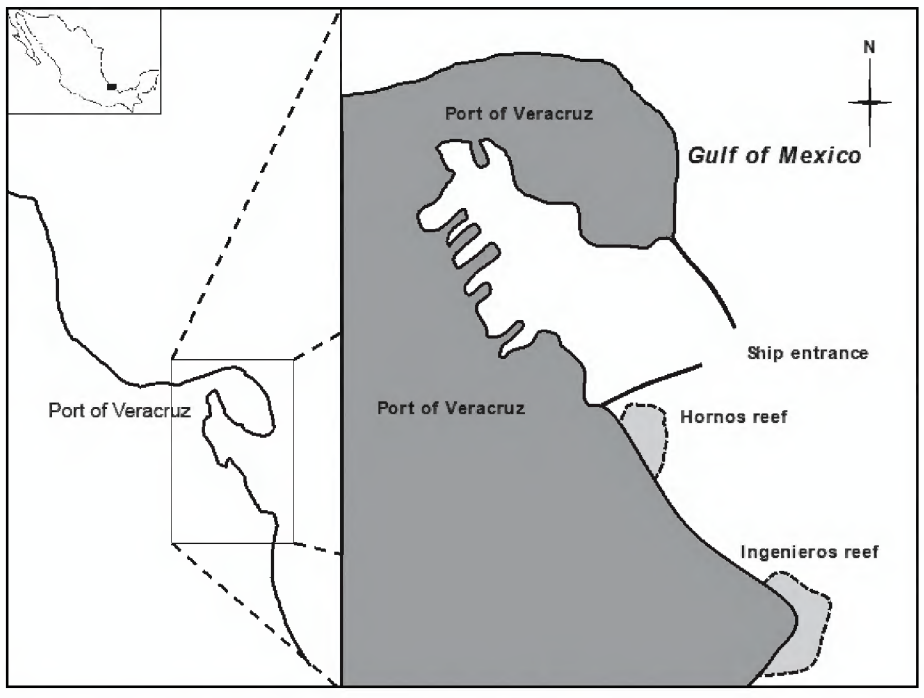

Figure 1. Map of the location of Hornos and Ingenieros reefs in the National Park Veracruz Reef System (NPVRS).

Bitar and Zibrowius 1997, Fine et al. 2001, Izquierdo et al. 2007, Sartoretto et al 2008, Laydet and Helberg 2015, Serrano et al. 2016, Brito et al. 2017).

Recently, several colonies of corals that have morphological characteristics similar to *O. patagonica* have been found in the National Park Veracruz Reef System (NPVRS) in the Gulf of Mexico. Our aim was to describe, for the first time, the presence of *O. patagonica* in the NPVRS on the coast of Veracruz, Mexico by carrying out a morphological analysis using the morphological characteristics of the colony and corallite and by a molecular analysis using the sequencing of the mitochondrial gene Cytochrome *b* (Cyt*b*) and nuclear gene *β* Tubulin.

Methods

Oculina specimens were collected from the reefs Hornos and Ingenieros at the (NPVRS) at 3 m depth (Fig. 1). Small fragments of about 5 cm^2 were collected. A small section of each fragment was preserved in ethanol (100%) for molecular analyses; the rest of the sample was used for morphological analysis. The sampling protocol of the NPVRS was approved by *Comisión Nacional de Acuacultura y Pesca* (*The National Commission of Aquaculture and Fishing*; permit no. PPF/DGOPA-072/13). None of the collected organisms are endangered or protected under Mexican law (NOM-059-SEMARNAT-2010). DNA extraction was performed using the Qiagen Kit for tissue and blood, following the instruction of the manufacturer. DNA quantity and quality were checked by electrophoresis in 1% agarose gel (80 V for 40 min).

The amplification was done with the following mitochondrial primers, to Cyt*b*: MCytbF 5′ GTT GCT AGT AGTAAT TTG GAT TG 3′ and MCytbR 5′ CAA ACC ACC CAA GCR RAA RA -3′; to Cytochrome Oxidase I: COIF and COIR; and to amplify nuclear *β tubulin*: TubulinF 5′ GCA GAA CGC TGG TCC TTA TTT-3′ and TubulinR 5′ ACA TCT GTT GTT CTG TGA GAG-3′. The protocol used for the amplification was 94 °C/120s, followed by 30 cycles at 94 °C for 45s, 58 °C for 45s, 72 °C for 90s, with a final phase of 72 °C for 5 min (Fukami et al. 2004).

The nucleotide sequences were obtained with a Genetic Analyzer 3130XL sequencer, and BigDye Terminator chemistry V3.1, with POP6 electrophoresis and run time of 2 h 30 min. The alignment of the sequences was performed with software Clustal X 2.1 (Thompson et al. 1997) using default parameters and the alignment was visually checked with Phyde v1.2. Subsequently, we executed a Basic Local Alignment Tool (BLAST) with the reference sequences reported in the genetic database to determine the corresponded taxonomic group.

The following closely related species were used as references for Cyt*b* sequences: *O. diffusa* AB117379.1.

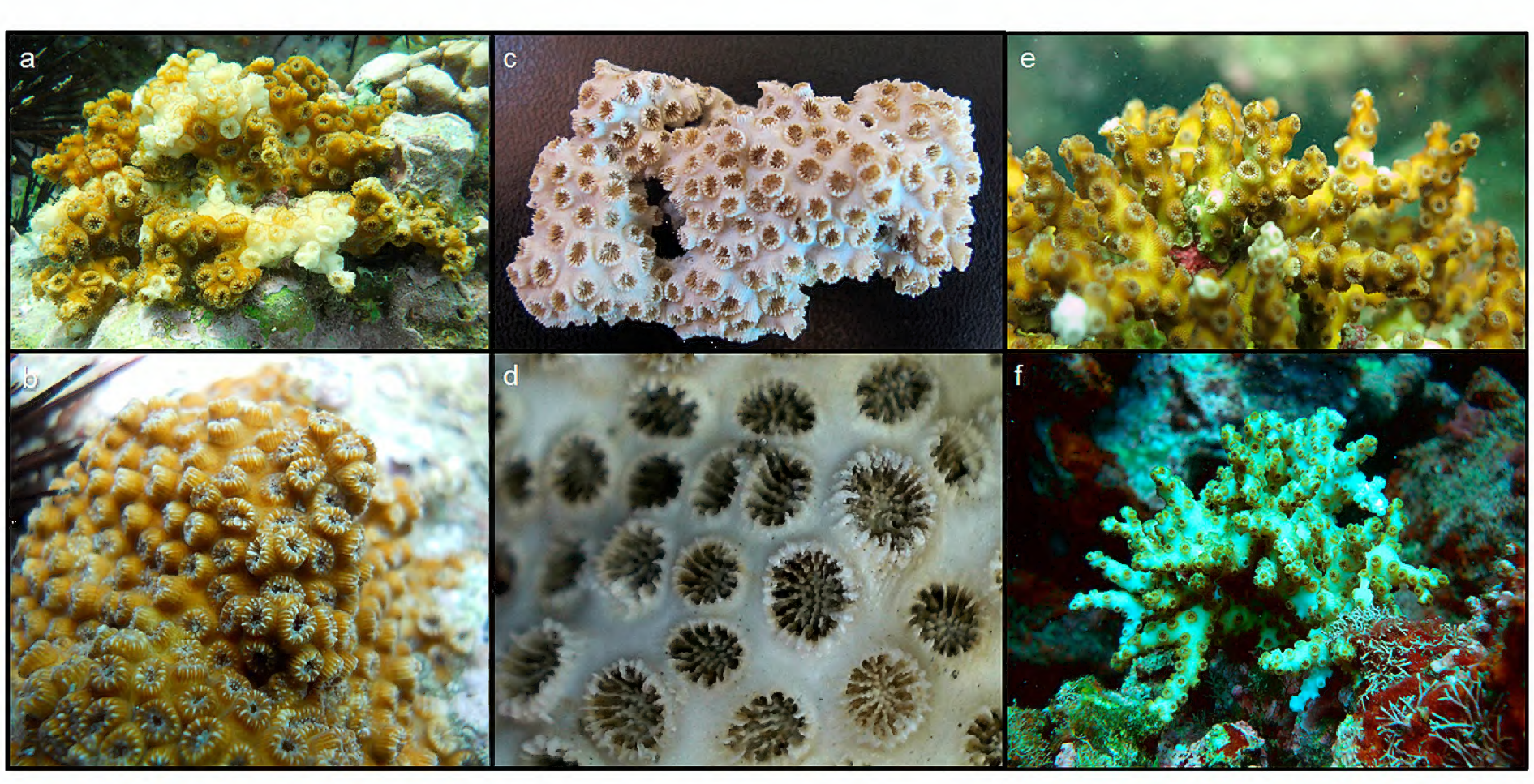

Figure 2. Colonies of *Oculina patagonica* in the NPVRS. **a, b.** Morphology of the colonies in the Hornos reef. **c, d.** Skeletal structure of the colonies without coral tissue from Ingenieros reef. **e, f.** *O. diffusa* from the NPVRS.

Accession numbers for *β tubulin*: *O. diffusa* AB118428.2; *O. patagonica* MG584231. For COI: *O. varicosa* FJ966868.1; *O. robusta* FJ 966869.1; *O. diffusa* FJ966871.1; *O. patagonica* LN614380.1.

For the morphological analysis, fragments of corals were washed with water. To remove soft tissue, the fragments were treated by submersion in boric acid 0.5% for 8 h. The cleaned fragments were dried in an oven at 90 °C for 2 days. Images were taken with a Phillips scanning electron microscope, model XL30ESEM. Samples were then covered with a layer of platinum for optimum resolution. The images were taken with a resolution of 3 nm with markers of 1– 2 mm and acceleration of 10– 20 kV. Morphometric analysis of each sample was conducted with the software Image J (Schneider 2012). To identify *O. patagonica* species we used as reference the morphological characteristics reported by Zibrowius (1974), several works (Fine and Loya 1995, Fine et al. 2001, Sartoreto et al. 2008) have used this classification to identify *O. patagonica* colonies (Fig. 2).

Results

New records. Mexico: Veracruz. Gallega Reef (19°13′ 34.66″ N, 096°07′39.95″ W), Jose L. Tello Musi coll.; September 2017. Hornos Reef (19°10′42.54″ N, 096°07′ 09.71″ W), Jose L. Tello Musi coll.; September 2017. Ingenieros Reef (19°09′07.42″ N, 096°05′28.91″ W), Jose L. Tello Musi coll.; September 2017. Hornos Reef (19°10′42.54″ N, 0 96°07′09.71″ W), Horacio Perez España coll.; September 2017.

Vouchers for each record are stored in the collection of the Laboratorio de Zoología, Universidad Nacional Autónoma de México, FES Iztacala.

Identification. We obtained the mitochondrial Cyt*b* and nuclear *β* tubulin sequences of the samples collected from the NPVRS. The length of the Cyt*b* sequences was 381 pb with an average G+C content of 29.9%. For *β* tubulin the length was 720 pb with an average G+C content of 43.1% and for COI the length was 356 pb with an average G+C content of 41%. The BLAST analysis was performed in the GenBank and EMBL-EBI European database. For COI we obtained a 100% similitude with *O. patagonica*, *O. varicose*, *O. robusta*, and *O. diffusa*, which suggests that proper identification of the species could not be made using only the COI gene. In the case of Cyt*b* and *β* tubulin there are no reported sequences of these genes for *O. patagonica* in both gene databases. The BLAST analyses in both databases found that the *O. diffusa* sequence (Fukami et al. 2004) is most similar, with a similitude of 99% and 98.7% for Cyt*b* and *β* tubulin, respectively.

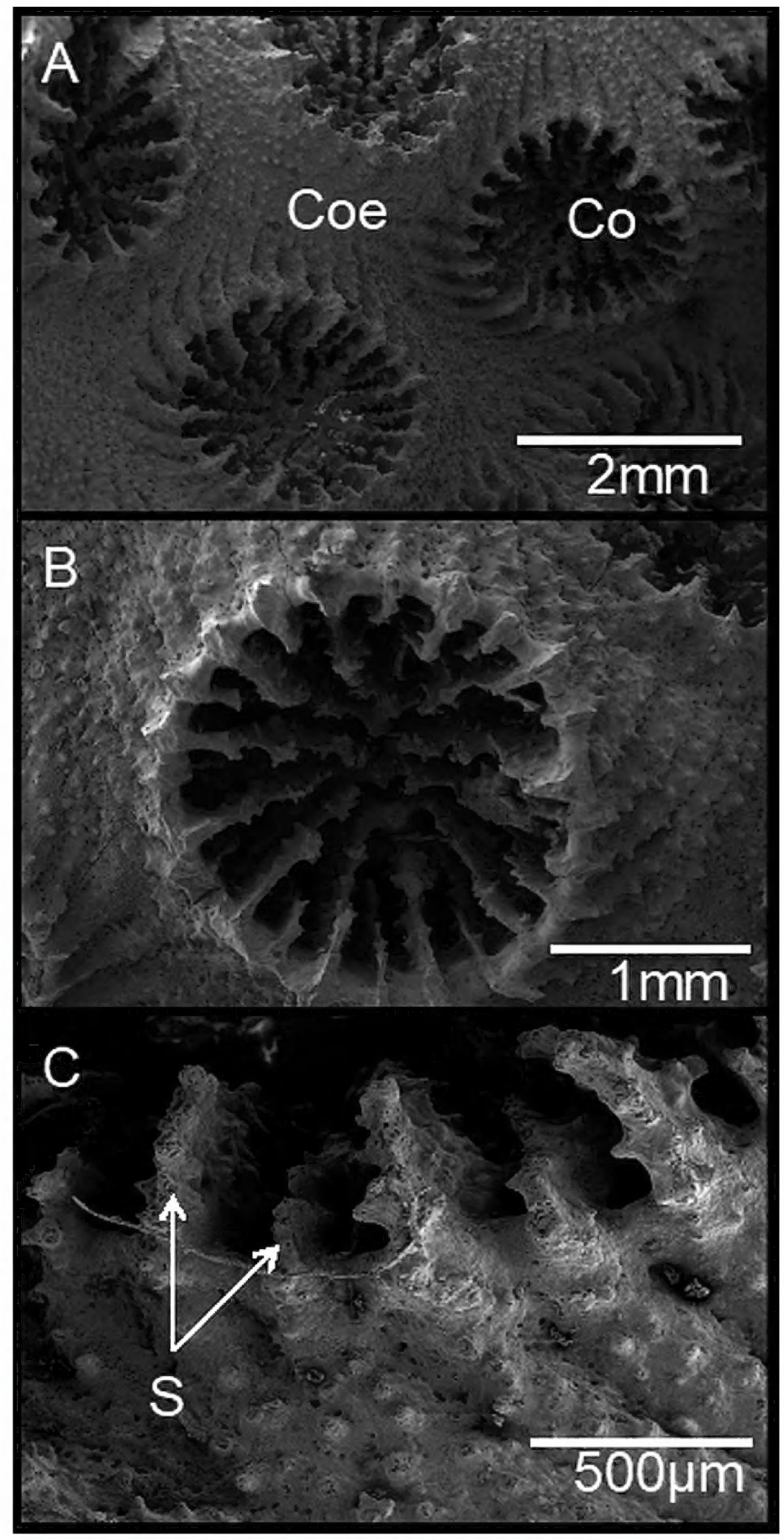

Figure 3. Images of the corallite of *Oculina patagonica* obtained with the SEM. **A.** Skeletal structure, corallite and coenosteum of the samples collected. **B.** Mophological characteristics of the corallite. **C.** Structure of the septa. Co = corallite; Coe = coenosteum; S = septa.

We found several colonies of *Oculina patagonica* in the Hornos, Ingenieros, Gallega, and Pajaros reefs at depths of 2–3 m. The colonies, with an approximate diameter of 30 cm, were encrusted and irregularly shaped, growing over rocky substrate; they varied from orange to brown. The corallites were 2–3 mm in diameter, rounded or elongated, with an average of 24 septa; 12 of the septa

Table 1. Morphological characteristics of *Oculina patagonica* reported by Zibrowius, (1974) and colonies found in the NPVRS.

	Colony form	Colony size	Calice form	Coenosteum	Corallite diameter	Septa number
O. patagonica (Zibrowius, 1974)	Encrusting	~ 40cm	Rounded or polygonal	Smooth or slightly wrinkled, with fine granulation,	~ 2–5 mm	24 (12 fused to center, 12 free)
O. patagonica form NPVRS	Encrusting	30cm	Rounded	Slightly wrinkled, with fine granulation	2–3 mm	24 (12 fused to center, 12 free)

were fused to the centre and the other 12 were unbounded (Table 1). They show a false columella formed by the union of long septa into a central axis. The coenosteum varies from smooth to wrinkled near the calice, marked by lines. Septa are located in the interior of the corallite. Long septa exhibit 1–3 teeth whereas short septa present no teeth at all (Figs 2, 3).

Discussion

Our morphological and molecular results confirm the presence of the invasive *O. patagonica* in the Gulf of Mexico for the first time. Based on morphological and molecular analyses using the COI (GenBank accession number: MH475366), Cytb (KY002687.1) and β tubulin (MH475367) the sequences of the *O. patagonica* specimens can be distinguished from *O. diffusa*, which is the most closely related species previously reported in the NPVRS (Horta Puga and Tello Musi 2009). *Oculina diffusa* in the NPVRS exhibit branched colony morphology (Fig. 2e, f), while colonies of *O. patagonica* in the NPVRS exhibit an encrusting morphology (Fig. 2a–d).

Oculina patagonica develops mainly in shallow waters, but it is capable of surviving in polluted and industrial habitats as well as in pristine conditions (Fine et al. 2002). This ability has allowed *O. patagonica* to greatly expand outside of its native range, the Mediterranean Sea, and spread to the Black Sea (Fine et al. 2001, Sartoreto et al. 2008). The introduction of *O. patagonica* in the Gulf of Mexico might have been caused by accidental transportation during the hull of ships or in ballast water (Zibrowius 1974, Sartoreto et al. 2008).

Hornos and Ingenieros reefs are located 2 km away of the Port of Veracruz (), and these reefs exhibit high rates of sedimentation and pollution, with the presence of several heavy metals (Mendel-Alvarado 2014) from urban and industrial discharges. These reefs are exposed to discharges of ballast water from commercial shipping through the NPVS to the Port of Veracruz. Shipping might be facilitating the arrival of *O. patagonica* in both reefs.

Colonies of *O. patagonica* have been observed in the Gulf of Mexico south of the NPVRS since 2015 (González Gándara et al. 2015), but methods for their identification were insufficient and the species was classified as *Oculina sp*. w SG) Colonies of *O. patagonica* reported by Gonzalez Gándara et al. (2005) suggest the capability of *O. patagonica* to adapt to varied environmental conditions in the Gulf of Mexico. The warm waters, space disponibility to settle new colonies, and biological characteristics such as stress tolerance, early reproduction, and fast growth of *O. patagonica*, might aid in the expansion of this species in the NPVRS. *Oculina patagonica* invades by propagating in areas where the coral reef habitat is modified by human activities (Lajeunse et al. 2010, Sacramento et al. 2013).

Acknowledgements

N. Colín García, expresses his gratitude to CONACyT for financial support. SEM pictures and EDXS analysis were performed at LANNBIO Cinvestav Mérida, which supported projects FOMIX-Yucatán 2008-108160 and CONACYT LAB-2009-01 No. 123913. We like to thank Alejandro Monsalvo Reyes for his help to obtain the gene sequences in the Bioquimica Molecular Laboratory in the UNAM FES Iztacala. We thank the Laboratorio Nacional de Ciencias de la Sostenibilidad (CONACYT LN280794), Laboratorio Nacional de Resiliencia Costera (CONACYT LN271544), Problemas Nacionales (CONACYT PN212745) and PAPIIT-DGAPA-UNAM (IN219515; IN220318) for financial support. We also like to thanks to the editor and reviewers of the manuscript.

Authors' Contributions

JLTM and HPE collected the samples, NACG and JLTM identified the samples, NACG, perform the morphological analysis, NACG and JECC perform the molecular analysis, NACG, JLTM, JECC and HPE wrote the text, JLTM and HPE found colonies in field, and NACG made the analysis.

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